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Abstract: Meiotic recombination is a specifically timed and regulated process which does not occur randomly throughout the genome, but tends to be clustered in 'hotspots'. There is extensive evidence that recombinatlon rate is influenced by chromatin conformation and that events are primarily initiated at gene promoter regions. In an effort to determine the pattern of chromatin condensation and recombinatlon at meiosis in an imprinted region, fine scale genetic mapping in the approximately 4 Mb Prader-Willi/Angelman syndrome deletion region was undertaken. The results Indicate that the male-female recombinatlon ratio can vary significantly over short regions. A male recombinatlon hotspot is localized to between the 3' end of GABRA5 and Dl 5S1 56, which Is adjacent to but outside the putative AS/PWS imprinted regions. In addition, a region of relatively high recombination In females is observed between Dl 5S1 28 and D15S97, which spans a domain of paternal allele-specific transcription implicated in the Prader-Willi syndrome. It is inferred that the Inactivation and relative condensation of this latter region on the maternal chromosome occurs as a post-meiotic modification

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Sex-specific meiotic recombination in the Prader–Willi/Angelman syndrome imprinted region

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Meiotic recombination is a specifically timed and regulated process which does not occur randomly throughout the genome, but tends to be clustered in 'hotspots'. There is extensive evidence that recombination rate is influenced by chromatin conformation and that events are primarily initiated at gene promoter regions. In an effort to determine the pattern of chromatin condensation and recombination at meiosis in an imprinted region, fine scale genetic mapping in the approximately 4 Mb Prader–Willi/Angelman syndrome deletion region was undertaken. The results indicate that the male–female recombination ratio can vary significantly over short regions. A male recombination hotspot is localized to between the 3′ end of GABRA5 and D15S156, which is adjacent to but outside the putative AS/PWS imprinted regions. In addition, a region of relatively high recombination in females is observed between D15S128 and D15S97, which spans a domain of paternal allele-specific transcription implicated in the Prader–Willi syndrome. It is inferred that the inactivation and relative condensation of this latter region on the maternal chromosome occurs as a post-meiotic modification.

INTRODUCTION

In the human genome, 1 Mb of DNA is equivalent, on average, to 1 cM (1% recombination) (1). Recombination does not occur randomly, however, but tends to cluster in 'hotspots', regions with relatively high recombination rates, separated by stretches of diminished recombination. The human female genetic map is about 50% longer than the male map; however, this is not a generalized reduction and recombination is higher in male meiosis for some specific intervals (2). There is also a striking sexual dimorphism in observed cytogenetic chromosome length, with human female pachytene chromosomes being approximately 50% longer than those in males (3). It thus seems likely that the 50% higher recombination in human females versus males is also a reflection of the more condensed state of male chromosomes.

A link between chromatin conformation and meiotic recombination in humans is also suggested by the observation that the recombinationally inactive sex chromosomes in male spermatocytes are highly methylated, condensed and transcriptionally inactive, whereas the X chromosomes in female oocytes, which do participate in recombination, are euchromatic and transcriptionally active (4). This has led to the suggestion that the specific heterochromatization of the sex chromosomes during meiosis in males is a means to prevent unwanted recombination (5). Further evidence for an influence of chromatin structure on recombination is supported by the virtual absence of any crossing-over in constitutive heterochromatin, which is both highly condensed and devoid of transcribed genes. In addition, hotspots for meiotic recombination in yeast and mice (which may be strain or sex-specific) have repeatedly been mapped to the sites of gene promoters or enhancers (6–11). In yeast it appears that all meiotic recombination, general and site-specific, may be initiated by double-strand breaks at promoter regions (11). Evidence suggests that it is an open chromatin conformation, and not gene transcription, that is necessary for recombination to occur at these hotspots (5,10,12,13).

Parental imprinting is the gamete-specific modification that distinguishes the maternal and paternal chromosomes in higher eukaryotes and may result in differential expression of genes depending on parent of origin (14). It has been hypothesized that male–female differences in recombinatory activity may be related to differences in gene activity during meiosis of the two sexes and specifically that regions activated in the imprinting process are potential sites for recombination (15). It has also been suggested that the process of homologous pairing and recombination may play a role in the modification of chromatin structure associated with imprinting (16). Other evidence suggests that the full imprint, as reflected by methyl-

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ation and chromatin structure in differentiated tissues, is probably not established until early in embryogenesis (14). However, differences in chromatin state established during meiosis may be involved in the 'marking' of a region as 'maternal' or 'paternal' prior to fertilization. Meiotic recombination associated with imprinted regions could therefore reflect this initial sex-specific imprinted state.

One of the most extensively studied examples of genomic imprinting is within human chromosome 15q11.2–12. Lack of a paternal copy of this region through deletion or maternal uniparental disomy results in the Prader–Willi syndrome (PWS) (17–20), whereas a maternal deletion or paternal uniparental disomy results in the completely distinct phenotype of Angelman syndrome (AS) (21,22). At least four genes (SNRPN, PAR-5, PAR-1 and IPW) have been identified in the PWS/AS deletion region which are expressed only on the paternal chromosome and which define an imprinted transcriptional domain of at least 200 kb (23,24). Maternal specific expression has not been observed; however, parent-of-origin specific replication timing (25,26), unusual deletions in PWS and AS patients (27,28) and linkage analysis (29–31) indicate that there is a region distal to the maternally imprinted PWS region which displays maternal allele-specific expression and is involved in the AS.

In an effort to determine the pattern of recombination and chromatin condensation at meiosis in an imprinted region, fine scale genetic mapping in 15q11–q13 in humans was undertaken. At least three distinct regions of sex-specific recombination are identified including a region of high female recombination which spans the PWS maternally imprinted domain, and a male recombination hotspot located between the 3′ end of the GABRA5 gene and D15S156. These data are compared with patterns of chromatin condensation observed in somatic cells and to physical mapping data.

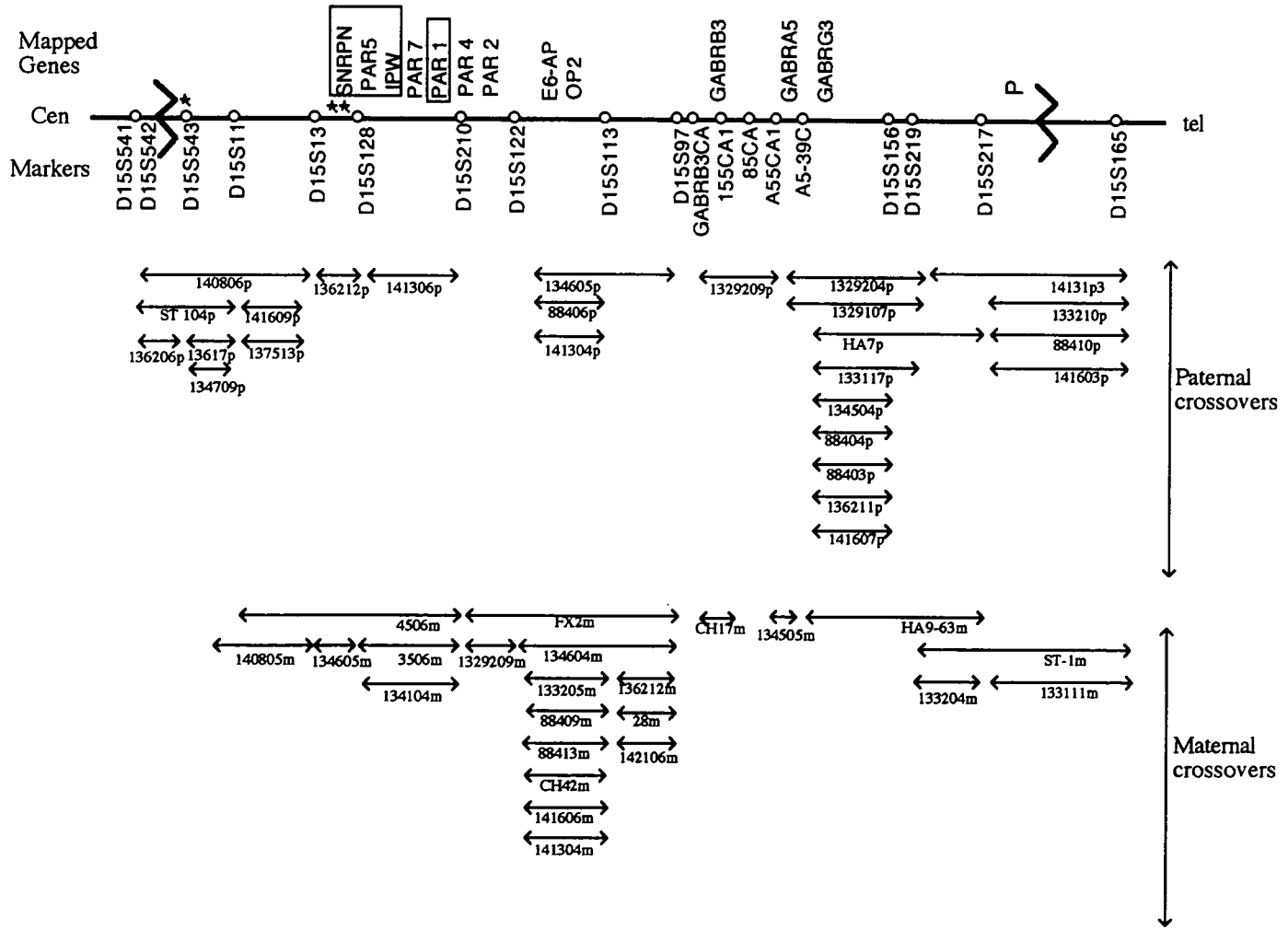

Figure 1. Map of crossovers localized between D15S541 and D15S165. Microsatellite loci are indicated along the top of the diagram. Each crossover is identified by the CEPH identification number or, in the case of previously published families (32), by a letter designation. Mapped genes are indicated above the microsatellite loci; those showing paternal-allele specific expression are enclosed in a box (23,24,33–36). Parent of origin specific methylation sites (37–39) are indicated by an asterisk. Distances are only approximate, as exact physical distances between most of these markers is not known.

RESULTS

In total, 49 crossovers were localized between D15S541 and D15S165 (26 paternal and 23 maternal). Twenty microsatellite markers were used to localize the crossover region in these families. Only the two parents, the recombinant individual, and one non-recombinant reference sib were typed for these additional markers. In most cases, multiple markers were informative on both sides of the crossover event to confirm that the crossover truly existed and was not due to a typing error. No double crossover events were observed within this region in any case, which could have indicated a typing error. A map of the minimum recombination region for each crossover is given in Figure 1. Lack of informativeness prevented further refinement of the crossover location in some cases.

A fine-scale genetic map of this region was constructed from the data based on total informative meioses used to identify crossovers within each specific interval (Fig. 2). Maximum interference was assumed for this, i.e. we assume that double crossovers were not likely for the intervals ascertained on, and therefore that all crossovers were identified.

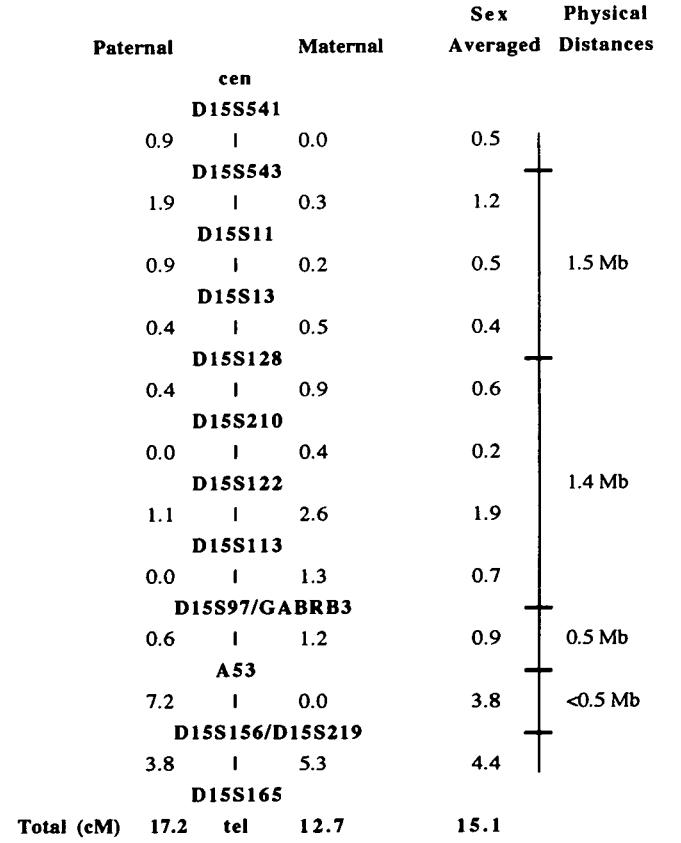

Figure 2. Sex-specific and sex-averaged genetic maps are derived from the present work (see text). Estimated genetic distances are given in percent recombination, which is equivalent to centimorgans if complete interference is assumed. Physical map distances are given for comparison and were derived as follows: for the D15S543 to D15S128 and D15S128 to D15S97 intervals, the previously published YAC contig of the region (33,40); for the D15S97 to A5–39CA and A5–39CA to D15S156 intervals, the physical map of the GABAA receptor subunit gene cluster (33,36,41). Note that for this last interval, 0.5 Mb is the best available estimate of maximum size.

Since the maximum genetic lengths of intervals under ascertainment on (D15S541 to GABRB3 and GABRB3 to D15S165) are less than 11 cM, this assumption is reasonable. When a crossover could not be assigned to a specific interval, the probability that it fell in one or another interval was estimated based on the other mapping data. For multiple overlapping potential crossover sites, an iterative procedure was used to estimate the location probabilities for each crossover. The distances on this map are quite similar to those from a previously published CEPH-based linkage map which reported female and male distances of 6.9 and 3.3 cM for D15S11 to D15S97/GABRB3; and 0.0 and 6.7cM for D15S97/GABRB3 to D15S156 respectively (42). The present data, however, indicate a slightly shorter distance from D15S156 to D15S165 than that previously reported. This estimate is, however, based on a relatively small number of informative meioses in both cases.

The genetic map indicates that male recombination exceeds female recombination for the interval from D15S541 to D15S13 (3.7 versus 0.5 cM) and from GABRA5 (3′ end) to D15S156 (7.2 versus 0 cM); whereas female recombination exceeds male recombination for D15S128 to GABRA5 (6.4 versus 2.1 cM) and D15S156 to D15S165 (5.3 versus 3.8 cM). The D15S128 to GABRA5 domain encompasses the majority of the currently known genes in this region (Fig. 1). D15S128 maps in the vicinity of SNRPN, the most centromeric of the four currently known maternally repressed/paternally expressed genes in 15q11–q13 (23,33).

In order to compare relative male to female recombination, the region was first divided into segments based on the markers initially used to screen families (D15S541, D15S11, D15S128, D15S97/GABRB3, D15S156 and D15S165). This was necessary to keep the number of informative chromosomes within a segment constant. Marker D15S156 was additionally chosen to further subdivide one segment because it appeared to delineate the boundary of a paternal high recombination region (data summarized in Table 1). Three of the six regions showed significant differences between male and female crossover rates, which corresponded to the two excess paternal and one of the excess maternal recombinatory regions noted from the genetic map (Table 1). Significant heterogeneity in male–female recombination between these three general regions was also shown using a contingency table analysis (Table 2).

Physical distances as best as they are known, are indicated for comparison on Figure 2. Overall, the approximately 4 Mb PWS/AS deletion region lies within a 15 cM genetic distance. There is a slight excess of male to female meiotic recombination (17.2 and 12.5 cM respectively), although there is 50% more female than male recombination in the genome as a whole. The greatest expansion of the genetic relative to physical map, where the sex-averaged recombination rate is more than seven times the genome average, occurs in the region of the paternal recombination hotspot distal to the 3′ end of GABRA5 and proximal to D15S156. The transition region between this hotspot and the high maternal recombinatory region contains the GABRB3 and GABRA5 genes and is characterized by a remarkably high frequency of highly polymorphic CA-repeat loci (only some of which are indicated on Figure 1) (43). It is also the location of a short maternal early-replicating domain within GABRA5 which is embedded in the much larger

Table 1. A comparison of male to female recombination frequency. Number of observed recombinants and total informative meioses are indicated for each interval

Total	D15S541–D15S11	D15S11–D15S128	D15S128–GABRB3	GABRB3–A5-39	A5-39–D15S156	A15S156–D15S165
Maternal observed	0–1 (132)	1–3 (280)	14–15 (280)	2 (159)	0–1 (159)	3–4 (76)
Paternal observed	4–5 (144)	3–4 (262)	4 (273)	1(161)	8–9 (161)	4–5 (114)
P (Fisher's Exact Test)[a]	n.s., 0.037	n.s.	0.012, 0.008	n.s.	0.031, 0.009	n.s.

[a]Two probabilities are given when the number of recombinants are variable: the first corresponds to the minimum possible difference and the second to maximum difference.

paternal early/maternal late replicating domain from SNRPN to GABRA5 (44).

DISCUSSION

The 3.5–4 Mb segment from D15S543 to D15S156 shows a sex-averaged genetic distance of approximately 10 cM, exceeding the 1 cM per 1 Mb average in the human genome. As recombination is known in other organisms to be specifically initiated at the sites of gene promoters, the rate of recombination may be influenced by gene density. This could indicate that the PWS/AS region contains a greater number of genes than the human genome average of one gene per 40–50 kb (45). As yet, only 13 genes have been identified within the common PWS/AS deletion region including SNRPN, PAR-1, PAR-5, IPW, PAR-7, PAR-4, PAR-2, OP2, HPEVE6A, GABRA3, GABRA5, GABRG3, and P (23,24,33–36). Since gene mapping efforts have concentrated primarily in two regions; around SNRPN and around the GABA receptor genes, there are likely to be many more genes in this region as yet unmapped. A possible correlation of recombination with gene density is, however, confounded by the sex specificity of the observed recombination. Clearly, there are secondary factors influencing recombination rate, which may be due, in part, to the packaging of the DNA into chromatin.

We have identified three domains of sex-specific meiotic recombination within the PWS/AS imprinted region. Whether or not sex-specific recombination is a characteristic of imprinted regions, the recombination data yield interesting information about chromatin state during meiosis. Comparison with chromatin state inferred from methylation, replication and gene expression in somatic cells can potentially indicate which regions are actively altered by the imprinting process after fertilization.

The region proximal to D15S11 shows an excess of paternal versus maternal recombination. No imprinted genes have been identified here, although the D15S9 locus (in the vicinity of D15S543) shows hypomethylation at several sites on the paternal chromosome (37). It is therefore most likely that this region is relatively decondensed on the male chromosome both during meiosis and in the mature embryo, with the reverse true for the female chromosome. The broad region (approximately 1400 kb) between D15S128 and D15S97/GABRB3 exhibits relatively high female recombination and is therefore presumed to be decondensed during maternal meiosis. In contrast, the maternally inherited chromosome in somatic tissues shows lack of transcription of the four known imprinted genes within the PWS critical region (23,24) and

Table 2. Pairwise comparison of sex ratio between intervals (assumes equal number of informative meioses for each sex)

	Maternal	Paternal
D15S541–D15S128	2–3	8
D15S128–A5-39	16–17	5
A5-39–D15S165	4	13

χ^2 = 12.66, df = 2, p <0.002 (minimizing differences between cells)
χ^2 = 14.78, df = 2, p <0.0001 (maximizing differences between cells)
Intervals from Table 1 were lumped in pairs due to minimum sample size requirements.

is also late-replicating (26), suggesting a highly condensed chromatin structure. Therefore it is hypothesized that it is not until after fertilization that the maternal copy of this region is actively converted to a more condensed 'imprinted' state. Deletion of a putative 'imprinting control locus' located proximal to SNRPN appears to result in abnormal inactivation of the paternal PWS region (23). A lack of the maternal copy of this 'control locus' has no phenotypic effect (23) and thus this PWS 'imprinting control locus' seems not to be involved in this post-zygotic inactivation of the maternal chromosome.

The most striking sex-specific deviations in recombination rates are observed in the less than 500 kb region just distal to GABRA5. This male hotspot of recombination is separated from the highest region of maternal recombination by the GABRB3 and GABRA5 gene cluster. It is intriguing that the sharpest transition from high female/low male recombination to very high male/very low female recombination would occur in the vicinity of a domain which is also characterized by sharp boundaries in transition of parent specific early/late timing of replication during the S phase (44). It appears that the basic genetic processes of recombination and replication are under remarkably fine-tuned control within this segment of the chromosome. Transcription, replication and recombination are not completely unrelated processes. They all involve very specifically timed and programmed events which are commonly initiated at the promoter regions of genes. This presumably involves a preferred ability of the involved factors to bind to, uncoil, and denature DNA at these sites. Although this region has not been directly implicated in PWS or AS, it has been shown that biparental inheritance of chromosome 15 is somehow necessary to establish the parent-of-origin specific replication timing observed within the GABRB3 and GABRA5 cluster (44). It is possible that an altered chromatin state distal to GABRA5 serves as a parent-of-origin 'tag' involved in the establishment of the replication imprint of the proximal adjacent region.

Although it is intriguing that the PWS/AS region displays sex-specific patterns of recombination, it is unlikely that that all regions showing sex-specific differences in recombination are associated with genomic imprinting. There are numerous male–female differences in genetic maps which may be due to fundamental differences in how chromatin is organized in oocytes versus spermatocytes, or due to differences in expression of genes involved in oogenesis and spermatogenesis. If, however, significant differences in sex-specific recombination are found to be one characteristic of regions showing imprinting, this may provide a strategy for searching for imprinted genes. Strong evidence suggests that there is at least one maternally imprinted gene on chromosome 7 associated with the Silver–Russell syndrome (46). The search for a putative growth factor gene on chromosome 7 could possibly be aided by screening regions showing the greatest sex-dependent deviations in recombination rate.

In addition, an awareness of regions subject to significant differences in male and female recombination rates are important for linkage studies. Recently, Fink *et al.* (47) reported linkage of autosomal spastic paraplegia to the PWS/AS region on 15q. Positive lod scores at zero recombination were observed for D15S128 and D15S156 with negative lod scores for D15S122 and D15S165. It is likely that this gene could be more accurately localized within this region by accounting for the strong sex-dependent nature of recombination, rather than assuming sex-averaged recombination for all intervals. A fine-scale analysis of recombination in other defined regions should prove useful for providing more accurate data for linkage studies, furthering our knowledge of the mechanism of recombination and gleaning useful information concerning the genomic organization during meiosis.

MATERIALS AND METHODS

Based on the publicly available on-line CEPH mapping data (version 1), parental haplotypes for markers from D15S11 to D15S165 were constructed. Segregation of these haplotypes was tracked within each family to identify crossover events. For most CEPH families (excepting the eight reference pedigrees), marker information was only available for D15S11, D15S97 and GABRB3. There is no observed recombination between D15S97 and GABRB3, and virtually all families were informative for one or both these markers. By typing for two polymorphisms (D15S541 and D15S542) proximal to D15S11 in a subset of CEPH families, additional crossovers between D15S541 and GABRB3 could be identified (these data have been submitted to the CEPH data base). The eight CEPH reference pedigrees had also been typed for additional markers within the deletion region distal to GABRB3 including D15S219 and D15S165. In addition, pedigrees which had already been typed for multiple proximal chromosome 15 markers for a previous study were used (32). A description of these pedigrees was given previously. In total, 49 crossovers were localized to this region (26 paternal and 23 maternal) from the original typing data. Since true double crossovers (i.e. excluding gene conversion events) are unlikely to occur over short distances, due to chiasma interference, it is unlikely that we have missed any crossover events using these markers.

Microsatellite polymorphisms were typed by the PCR using standard techniques. PCR amplification was performed on a Perkin Elmer Thermocyler with 30–32 cycles of 1 min at 94°C denaturation, 1 min at 55–57°C annealing and 1–2 min at 72°C extension temperatures. 0.5–3 ml of reaction was mixed with an equal volume of urea loading buffer (42% urea, 0.1% xylene cyanol, 0.1% Bromphenol blue and 0.1% of 0.5 M EDTA) and directly loaded on to a 0.4 mm thick 6% polyacrylamide/50% urea gel. Visualization of bands was done by silver staining of the gels.

Information on all primers is available from the Genome Data Base; see also refs 33, 40 and 43). Microsatellite loci used are indicated in Figure 1. Only a few of these markers have been included in published genetic maps and this information is presented also in Figure 1. Recombination estimates for D15S541 to D15S11 are based on the present. Physical map data and PWS/AS critical region information were inferred from several sources (23,28,40,43,48). Individuals showing a recombinant haplotype are indicated on Figure 1 by either the CEPH identification number or, in the case of previously published families (32), by a letter designation.

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